



Distribution and bacterial availability of dissolved neutral sugars in the South East Pacific

R. Sempéré, M. Tedetti, Christos Panagiotopoulos, B. Charrière, France van Wambeke

► To cite this version:

R. Sempéré, M. Tedetti, Christos Panagiotopoulos, B. Charrière, France van Wambeke. Distribution and bacterial availability of dissolved neutral sugars in the South East Pacific. 2008. hal-00330286

HAL Id: hal-00330286

<https://hal.science/hal-00330286>

Preprint submitted on 14 Oct 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biogeosciences Discussions is the access reviewed discussion forum of *Biogeosciences*

Distribution and bacterial availability of dissolved neutral sugars in the South East Pacific

R. Sempéré, M. Tedetti, C. Panagiotopoulos, B. Charrière, and F. Van Wambeke

Laboratoire de Microbiologie, Géochimie et Ecologie Marines (LMGEM), UMR CNRS/INSU 6117, Case 901, Centre d'Océanologie de Marseille, Université de la Méditerranée, 13 288 Marseille Cedex 9 France

Received: 3 December 2007 – Accepted: 14 December 2007 – Published: 14 February 2008

Correspondence to: R. Sempéré (richard.sempere@univmed.fr)

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

Abstract

The distribution and bacterial availability of dissolved neutral sugars were studied in the South East Pacific from October to December 2004 during the BIOSOPE cruise. Four contrasted sites were investigated: Marquesas Islands (MAR), the hyper-oligotrophic South Pacific Gyre (GYR), the eastern part of the Gyre (EGY), and the coastal waters associated to upwelling of Chile (UPW). Total (free and combined) dissolved neutral sugar (TDNS) concentrations were higher in UPW (149–329 nM) and MAR (111–540 nM), than in GYR (79–390 nM) and EGY (58–492 nM). Nevertheless, their contribution to dissolved organic carbon (TDNS-C/DOC%) was generally low for all sites varying from 0.5% to 4% indicating that our South East Pacific surface waters were relatively poor in neutral sugars. Free dissolved neutral sugar (FDNS; e.g. sugars analyzed without hydrolysis) concentrations were very low within the detection of our method (5–10 nM) accounting <5% of the TDNS. In general, the predominant sugars within the TDNS pool were glucose, xylose, arabinose, and galactose while in the FDNS pool only glucose was present. TDNS stock to bacterial production ratios (integrated values from the surface to the deep chlorophyll maximum) were relatively high in GYR with respect to the low primary production, whereas the opposite trend was observed in the highly productive area of UPW. Intermediate situations were observed for MAR and EGY. Bioavailability of dissolved organic matter (DOM) exposed to natural solar radiation was also experimentally studied and compared to dark treatments. Our results showed no or little detectable effect of sunlight on DOM bacterial assimilation in UPW and in GYR while a significant stimulation was found in MAR and EGY. The overall results clearly suggest the semi-labile character of DOM in GYR compared to the labile of UPW and are consistent with dissolved organic carbon accumulation and the elevated C/N ratios reported by Raimbault et al. (2007).

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

1 Introduction

Sugars are among the most abundant components in seawater constituting structural and storage compounds (Painter, 1983; Parsons et al., 1984; Benner and Kaiser, 2003) of marine organisms and generally account 20–40 dry wt. % of plankton, 17 dry wt. % of bacteria (Stouthamer, 1977), 2–30% of dissolved organic matter (DOM; Pakulski and Benner, 1994; Benner, 2002), and 50–70% of the high molecular weight DOM (>1 kDa; Benner et al., 1992; Aluwihare et al., 1997). Two major categories of sugars have been identified in DOM: Monosaccharides (free monomers) and polysaccharides (neutral sugars released after acid hydrolysis). Concentrations of free sugars have been found extremely low (<50 nM) compared to the dissolved polysaccharides (200–800 nM) (Benner, 2002 and references therein). Of the free sugars glucose appears to be quantitatively most important although concentrations of arabinose and fructose have also been reported (Rich et al., 1996, 1997; Skoog et al., 1999; Kirchman et al., 2001). Only few vertical profiles of dissolved sugars in seawater have been already published (Skoog and Benner, 1997; Amon and Benner, 2003) and there is a limited number of studies dealing with the bacterial reactivity of polysaccharides and monosaccharides (free glucose) in the water column for contrasted trophic regimes (Rich et al., 1996; Skoog et al., 1999; Kirchman et al., 2001).

The South East Pacific waters encompass a wide range of primary productivity ranging from the most oligotrophic and clearest waters of the world ocean in the central part of the South Pacific Gyre (SPG) (Claustre and Maritorena, 2003; Morel et al., 2007; Tedetti et al., 2007) towards the highly eutrophic area of the upwelling of Chile (Carr, 2002). However, very little is known about DOM dynamics in the South Pacific, whereas there is no report dealing with sugar distribution in the SPG. Such a study could help to evaluate DOM characteristics in terms of bacterial cycling and abiotic degradation (solar radiation).

This paper aims to provide data on the molecular distribution of sugars in the DOM pool in the South East Pacific waters at four contrasted sites and to understand its

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

photochemical and bacterial reactivity.

2 Material and methods

2.1 Sampling

Sampling was performed along a 8000 km transect in the South East Pacific during the Biogeochemistry and Optics South Pacific Experiment (BIOSOPE) cruise (24 October–11 December 2004). To study the molecular distribution and bacterial availability of dissolved neutral sugars, we collected (1) depth profile samples (0–500 m) and (2) large volume samples (20 l) for DOM-photodegradation and -biodegradation experiments. Four stations were sampled: Marquesas Islands (MAR), center of the SPG (GYR), East of the SPG (EGY), and the upwelling of Chile (UPW) (Fig. 1; Table 1). Biogeochemical and physical characteristics of these sampling sites are described in detail elsewhere (Claustre et al., 2008). At these four long stations were also performed surface and underwater solar irradiance measurements (see below).

Discrete seawater samples were taken with 12 l Niskin bottles mounted on a CTD/rosette and washed with 1 M HCl and Milli-Q water before the cruise. Rubber-made ribbons and o-rings of the original Niskin bottles were replaced with silicon ribbons and Viton o-rings, respectively. Samples for dissolved sugar and bacterial production (BP) measurement were collected at six depths (0–500 m) close to solar noon (bio-optical CTD cast) directly (without tubing) from the Niskin bottles into precombusted (450°C, 6 h) 500-ml glass bottles, first or after gas sampling to avoid organic carbon contamination. The bottles were rinsed two times with the respective water samples before filling. Following collection, seawater for dissolved sugar analysis was filtered through 0.2-µm polycarbonate filters (Nuclepore, 47 mm filter diameter) which were washed with a few ml of 1 M HCl, 2 l of Milli-Q water, and 150 ml of sample prior to filtration. Samples were transferred to 5 ml polypropylene tubes (prewashed with 1 M HCl and Milli-Q water) and stored in the dark at –18°C. BP measurements

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

were made on unfiltered seawater. For DOM-photodegradation and -biodegradation experiments, samples were collected at two depths (5 m and deep chlorophyll maximum (DCM), except in MAR: 5 and 160 m) at midnight in 20 l Nalgene carboy bottles using Teflon tubing. Samples were immediately processed in a temperature controlled laboratory on board. Plastic gloves were worn and care was taken to minimize contamination during sampling and the following procedure. Glassware filtration material was precombusted before the cruise and rinsed with 1 M HCl and Milli-Q water after each sample. Sampling information is summarized in Table 1.

2.2 DOM-photodegradation and -biodegradation experiments

After sampling, seawater (from 5 m and DCM) was filtered through precombusted GF/A filters (Whatman, 142 mm filter diameter) using a peristaltic pump with acid-cleaned silicon tubing and then through acid-cleaned Whatman POLYCAP AS 0.2 μm (820-cm²) filter capsules to exclude bacteria and others microorganisms. Seawater was also filtered under a low vacuum (<50 mm Hg) through 0.8 μm polycarbonate filters (Nuclepore, 47 mm filter diameter) to prepare the bacterial inoculum whilst excluding predators. The filters were washed with 150 ml of 1 M HCl (filter capsule and Nuclepore), 2 l of Milli-Q water and 150 ml of sample (filter capsule, Nuclepore and GF/A) prior to filtration. The 0.2 μm filtered seawater (here after called DOM-solution) was distributed into precombusted 5 l glass bottles and stored at 4°C in the dark until morning. DOM-solutions were transferred into 100 ml quartz and Pyrex Winkler flasks and exposed on the ship deck for one day (~8 h around solar noon) to natural solar radiation in a recirculating water bath (0.1 m depth) to minimize temperature fluctuation. Average temperatures ranged from 27.5°C (MAR) to 14.5°C (UPW) (Table 1). Samples were irradiated in duplicates for two light conditions: so-called “Full Sun” (FS: total solar radiation in quartz flasks) and “Dark” (Pyrex flasks wrapped with aluminum foil). Before and directly after irradiation of DOM-solutions, aliquots were taken in FS and Dark samples for free dissolved neutral sugar (FDNS) measurements and stored in 5 ml polypropylene tubes (prewashed with 1 M HCl and Milli-Q water) in the dark at

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

–18°C. During DOM-photodegradation, the bacterial inoculum was kept in the dark at in situ temperature. Quartz and Pyrex flasks were precombusted before the cruise and extensively washed with 1 M HCl and Milli-Q water between different sets of samples during the cruise.

5 Bacterial response to the DOM lability change after photodegradation was quantified through biodegradation experiments. After irradiation, the DOM-solutions were mixed with the (unirradiated) bacterial inocula (1/6, inoculum/DOM-solution final ratio) and then dispensed in duplicate into several precombusted 100-ml Pyrex Winkler flasks and incubated in the dark at in situ temperature. No nutrients were added in the mixed
10 solutions in order to measure the response of bacteria to “natural” conditions. Samples were analyzed for BP at time 0, 24, 48, and 72 h of incubation. For each experiment, killed controls were made by addition of HgCl₂ (final concentration: 10 mg l⁻¹), incubated with other samples and analyzed at the end of the experiment for BP.

2.3 Total dissolved neutral sugars (TDNS)

15 After a quick defrosting, samples (4 ml) for total (free and combined) dissolved neutral sugar (TDNS) analysis were transferred into precombusted Pyrex vials and hydrolyzed under N₂ with 0.1 M HCl at 100°C for 20 h (Burney and Sieburth, 1977). After hydrolysis, samples were not neutralized (to avoid contaminations from the addition of calcium carbonate) but directly desalted using AG2-X8 and AG50W-X8 Bio-Rad ion
20 exchange resins according to Mopper et al. (1992). The reactions between resins and acidified samples (pH 1) favored the elimination of carbonates (which were released into the sample by anionic exchange with chloride) into CO₂, allowing a partial neutralization of sugar samples (pH 3–4.5 after desalting). A portion of the initial sample was also directly desalted (see above) without hydrolysis in order to estimate the amount of
25 FDNS.

The samples were injected into a WATERS-HPLC system through a manual Rheodyne valve equipped with a 200 µl sample loop. The mobile phase consisted of a mixture 95/5 (v/v) of low-carbonate NaOH (20 mM, Baker) and Milli-Q water which was

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

pumped at a flow rate of 0.7 ml min^{-1} on isocratic mode. Neutral sugars were separated on a Dionex Carbpac PA-1 anion exchange column ($4 \times 250 \text{ mm}$) with 19 mM NaOH at 17°C (column temperature) and were detected by a Decade electrochemical detector (Antec Leyden BV) using a gold working electrode and a Pd reference electrode (Panagiotopoulos et al., 2001; Panagiotopoulos and Sempéré, 2005). A 1 M NaOH solution was added to the eluent stream by a post-column pump at a flow rate of 0.2 ml min^{-1} to increase detector sensitivity. To avoid absorption of carbonates by NaOH, eluent solutions were degassed before use and constantly purged with helium at a flow rate of 4 ml min^{-1} .

Recoveries of desalting, estimated in spiked ($20\text{--}100 \text{ nM}$) sodium chloride solutions ranged from 72 to 80% for fucose, rhamnose, arabinose, mannose, xylose and ribose, and between 85–100% for galactose and glucose, respectively. Procedural blanks run with desalted sodium chloride solutions, showed only small peak of glucose ($\sim 5 \text{ nM}$), even though a systematic peak induced by desalting was coeluted with fructose avoiding its quantification. The detection limit was $5\text{--}10 \text{ nM}$ for all sugars. Analytical errors determined from duplicate analysis were $<8\%$ for all sugars except ribose (15%). Concentrations of dissolved sugars presented in this study are corrected for the blank values (glucose)

2.4 Bacterial production (BP)

BP was estimated from the incorporation rate of ^3H -leucine using the centrifugation method (Smith and Azam, 1992). The detailed protocol is fully described in Van Wambeke et al. (2008a). Briefly, 1.5 ml duplicate samples were incubated in the dark for $1\text{--}2 \text{ h}$ with 20 nM addition of leucine. Error associated to the variability between replicate measurements (half the difference between the two replicates) averaged 13 and 6% for BP values less and more than $6.7 \text{ pmol leu l}^{-1} \text{ h}^{-1}$, respectively.

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

2.5 Surface and underwater solar irradiance

Two profiles of downward irradiance were made at each station (Fig. 1) close to solar noon using a Satlantic MicroPro free-fall profiler equipped with OCR-504 downward irradiance in the UVB (305 nm), UVA (325, 340 and 380 nm), and visible (412, 443, 490 and 565 nm) spectral domains. Surface irradiance was simultaneously measured at the same channels on the ship deck using other OCR-504 irradiance sensors to account for the variations of cloud conditions during the cast, as well as to monitor UV and visible irradiances during the exposure period for the DOM-photodegradation experiments. A detailed description of optical measurements and determination of 10% irradiance depth ($Z_{10\%}$ in m) is given elsewhere (Tedetti et al., 2007).

3 Results and discussion

3.1 General observations

The main biogeochemical characteristics of the four stations are presented in Table 1. DCM ranged from 40 m (UPW) to 175 m (GYR) and was very close to the corresponding depth of euphotic zone [Z_{eu} , the depth of 1% surface photosynthetically available radiation (PAR)] Surface chlorophyll-*a* (Chl-*a*) concentrations as well as BP and primary production (PP) integrated down to DCM (I-BP and I-PP) showed large variations between the stations. In the GYR station, Chl-*a*, I-BP and I-PP values were low compared to the other stations (Table 1) which further confirms its hyper-oligotrophic status. In this area, PP was strongly nutrient-limited because of the absence of terrestrial inputs and because of the depth of nutricline ($0.01 \mu\text{M N}$ at ~ 160 m, Raimbault et al., 2007). In the MAR station, Chl-*a* (0.32 and $0.43 \mu\text{g l}^{-1}$ within surface and at the DCM, respectively), and I-PP ($683 \text{ mg C m}^{-2} \text{ d}^{-1}$) values were higher than those previously observed in this high nutrients low chlorophyll area (Signorini et al., 1999), probably due to vertical inputs of dissolved iron from subsurface waters (Blain et al., 2007).

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

From October to March, wind-driven coastal upwelling fertilizes the surface waters off Chile leading to one of the most productive areas in the world ocean (Carr, 2002). In the same way, $Z_{10\%}$ in the UVB, UVA, and visible spectral domains showed great variability with the highest values recorded in GYR (21 m at 305 nm to 113 m at 443 nm) and the lowest values measured in UPW (3 m at 305 nm to 18 m at 565 nm) (Table 2). The values reported in GYR suggest that this area likely contains the clearest oceanic waters of the world ocean (Morel et al., 2007; Tedetti et al., 2007).

3.2 Concentration levels of TDNS and FDNS

Concentrations of TDNS (free and combined) in depth profiles ranged from 50 nM (GYR, 40 m) to 834 nM (MAR, 30 m) and were generally higher above or close to the DCM, which probably reflects the high contribution from autotrophic organisms from cellular release processes (Fig. 2). Two general trends can be observed in a first approach: (1) the depth profile patterns generally followed those of Chl-*a* for MAR and EGY and, (2) TDNS concentrations at the highly eutrophic site of UPW were moderately elevated (131–289 nM) and lower than that observed in the highly oligotrophic GYR (50–389 nM). This feature was kind of surprising because Chl-*a* and I-PP values were one order of magnitude higher in UPW than in GYR (Fig. 2; Table 1). Similar TDNS concentrations in the upper 500 m (180–800 nM) have been already observed in Equatorial Pacific (Skoog and Benner, 1997), Northeastern Pacific and Sargasso Sea (Borch and Kirchman, 1997), Central Arctic (Rich et al., 1997), Ross Sea (Kirchman et al., 2001), as well as in ultrafiltrated DOM samples (20–661 nM) collected from Equatorial Pacific and Arctic Oceans (Skoog and Benner, 1997; Amon and Benner, 2003). It is worth to notice that higher sugar concentrations (1000–4000 nM) above the thermocline were reported only for coastal samples (e.g. inshore the Oregon coast; Borch and Kirchman, 1997, Arctic estuarine samples; Amon and Benner, 2003). Our TDNS concentration results are not comparable to those obtained by colorimetric techniques [3-methyl-2-benzothiazolinone hydrazone (MBTH) or 2,4,6-tripyridyl-s-triazine (TPTZ)] because these techniques include in their analysis a much broader spectrum of sug-

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

ars including methylsugars, aminosugars, uronic acids etc. These compounds were outside the analytical window of the HPAEC-PAD technique under our current analytical conditions (Borch and Kirchman, 1997; Skoog and Benner, 1997; Panagiotopoulos and Sempéré, 2005).

5 Although our sugar measurements were made only in the upper layers and we do not have full profile data, we expect that sugar concentrations will decrease with depth. Reported literature data below the thermocline ranged from 90 to 450 nM for Northeastern Pacific, Equatorial Pacific and Sargasso Sea (Borch and Kirchman, 1997; Skoog and Benner, 1997), and from 31–68 nM for deep Arctic water samples (Amon and Benner, 2003). These results are in good agreement with our lowest TDNS concentrations (58 nM for EGY-250 m and 185 nM for GYR-270 m). FDNS concentrations were within the detection limit of the PAD (5–10 nM) in most of the sites and ranged from undetectable to 35 nM (MAR, 30 m). Our results are much lower than those found in the Equatorial Pacific (20–110 nM, Rich et al., 1996), and Central Arctic (31–68 nM; Rich et al., 1997) but similar to that reported in Gulf of Mexico (3–7 nM; Skoog et al., 1999) and Ross Sea (0–14 nM; Kirchman et al., 2001). FDNS comprised a small fraction of TDNS in all stations and generally were <5% of the TDNS.

3.3 TDNS and FDNS yields

20 TDNS yields were calculated by dividing TDNS-C by dissolved organic carbon (DOC) (Raimbault et al., 2007) and as such are presented as a percentage of DOC (TDNS-C \times DOC⁻¹%). TDNS yields ranged from 0.5 to 4% with higher values recorded below the DCM. Higher TDNS yields (0–80 m, mean=3.32%) were measured in MAR which probably reflects a terrestrial influence. TDNS yields correlated well with TDNS concentrations ($n=23$, $r^2=0.94$, $p<0.001$) but not with salinity ($n=23$, $r^2=0.038$, $p>0.1$) and DOC ($n=23$, $r^2=0.13$, $p>0.01$) indicating that neutral sugar yield is a useful indicator of the phytoplankton-derived DOM (Amon and Benner, 2003). Our results are in good agreement with previous values reported in the Equatorial Pacific (1–7%, Rich et al., 1996; 2–6%, Skoog and Benner, 1997), Ross Sea (1–11%, Kirchman et al., 2001),

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

and Central Arctic (2–20%, Rich et al., 1997).

3.4 Molecular composition of TDNS and FDNS

Three major sugar classes were detected in South East Pacific samples including the aldohexoses (glucose, galactose, and mannose), deoxysugars (fucose, and rhamnose), and aldopentoses (arabinose, and xylose). Concentrations of fructose (ketohexose) and ribose (aldopentose) are not reported here because they were very low and often not detected (Fig. 3). Aldohexoses were in most of the sites the most abundant compound class (30–727 nM) followed by aldopentoses (27–131 nM), and deoxysugars (<10–91 nM). Glucose was very often the most abundant (15–77% of the TDNS) followed by xylose (7–40%), arabinose (4–24%), and galactose (3–44%). Mannose, fucose and rhamnose were less abundant accounting <10% of the total sugar pool. Note that xylose was predominant at 40 and 90 m in GYR, and at 5 m in EGY stations, whereas arabinose was predominant in UPW in the first 0–25 m (Fig. 3).

Our results indicated that glucose was clearly the most abundant sugar at greater depths, and that its relative abundance increase with depth in GYR and in EGY (Fig. 3). The dominance of glucose within the TDNS pool was reported from several oceanic regions including the Equatorial Pacific (0–4000 m, 21–61%; Skoog and Benner, 1997), Sargasso Sea (surface, 35%; Borch and Kirchman, 1997), Central Arctic (0–40 m, 39% Rich et al., 1997), and Antarctic (0–60 m, 35–37%; Kirchman et al., 2001). Glucose was also the only detectable sugar within the FDNS pool with concentrations ranging from 2 to 10 nM in most of the sites (see above). The 100% abundance of glucose in the FDNS pool for our South East Pacific samples is in agreement to that reported in Gulf of Mexico (Skoog et al., 1999), and Ross Sea (Kirchman et al., 2001). Other studies carried out in the Equatorial Pacific and central Arctic indicated that glucose (47–79%), fructose (15–16%), and arabinose (5–15%) were present in the FDNS pool (Rich et al., 1996; Rich et al., 1997).

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

3.5 Sugar stocks in relation with biological activity

TDNS stocks integrated to the DCM ranged from 591 (UPW) to 2190 mg C m⁻² (MAR). Intermediate values were found in GYR and EGY (1651 and 1439 mg C m⁻²). Note that DCMs are considerably different according to the stations studied, and then higher stocks need to be related to the depth of the euphotic layer. However, these results indicate that sugars are relatively abundant in the euphotic layer regarding the bacterial activity and the high I-BP/I-PP ratio in the SPG (GYR) and on the border (EGY). Because sugars are by-products of PP and are essentially consumed by bacteria, our results indicate large variability across the transect between dissolved sugars production by PP and derived processes and its utilization by bacteria. Interestingly, the results indicate that MAR and UPW are characterized by high PP, relatively low I-BP/PP ratios and high and low dissolved sugars pools (2190 and 591 mg C m⁻², respectively). On the other hand, GYR and EGY are characterized by relatively elevated sugar stocks regarding the low I-PP.

Excess-dissolved sugars in these waters might be explained by the accumulation of recalcitrant sugars due to rapid diagenetic processes governed by microorganisms and/or to nutrient deficiency which results to low bacterial activity. Indeed, TDNS/BP ratios, which might be considered as representative of the sugar reactivity, varied considerably between the stations studied (Fig. 4). Our results clearly showed low TDNS/BP ratios in highly productive area (UPW, MAR) suggesting that although significantly abundant, TDNS are rapidly exhausted by bacterial consumption. On the other hand, in the less productive areas (GYR and EGY), TDNS are relatively abundant with respect to BP, and therefore accumulate in the surface layer. These results are consistent with Raimbault et al. (2007) observations indicating high concentration of DOC as well as elevated C/N ratios (GYR: 16–18; EGY: 13–19) compared to MAR (12–15). Such feature might be related to a malfunctioning of the microbial loop with severely nutrient limited heterotrophic bacteria (Thingstad et al., 1997). Indeed, Van Wambeke et al. (2007) using enrichment experiments, reported clear BP limitation by nitrogen in

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

GYR. Because of low horizontal advection as well as low seasonal convective mixing in GYR (Raimbault et al., 2007), BP nitrogen limitation is likely to be predominant all over the year (Van Wambeke et al., 2007), leading to an accumulation of sugar-rich DOC in surface waters.

5 3.6 Bacterial responses to DOM photodegradation

Solar doses (surface irradiances integrated over time) received by the DOM-solutions during the photodegradation experiments are presented in Table 3. Doses were higher in MAR and UPW (sunny days), ranging from ~2.2 (305 nm) to 52.7 kJ m⁻² (490 nm), than in GYR and EGY (cloudy days), ranging from ~1.3 (305 nm) to 27.5 kJ m⁻² (490 nm) (Table 3). A complete assessment of solar UV doses is provided in Van Wambeke et al. (2008b). DOM-photodegradation and -biodegradation experiments indicated contrasted effects of solar radiation on the DOM bioavailability to heterotrophic bacteria (Fig. 5). In MAR and EGY, surface DOM photodegradation led to a stimulation of BP by 150 and 133%, respectively (FS compared to Dark). However, the photodegradation of DOM collected deeper (180 m in MAR and 80 m in EGY) increased BP only in EGY (by 80%) (Fig. 5). Van Wambeke et al. (2007) found that heterotrophic bacteria were not bottom-up controlled in MAR, whereas they were limited by labile DOC (e.g. glucose) in EGY. We did not detect any significant photochemical production of FDNS during the photodegradation experiments. Therefore, the stimulation of BP after the DOM irradiation may be explained by the photochemical production of (other) bioavailable low molecular weight organic compounds such as aldehydes or organic acids that have been shown to be the major DOM-derived photoproducts (Kieber et al., 1990; Moran and Zepp, 1997; Mopper and Kieber, 2002). In GYR, surface DOM photodegradation did not significantly change BP. This was maybe due to the very low content in chromophoric (photoreactive) DOM in surface waters of the SPG (Morel et al., 2007) that prevents any photochemical process to occur. On the other hand, Van Wambeke et al. (2007) observed that in GYR, BP was limited by nitrogen and not labile DOC. Thus, even though some organic photoproducts might have been released from

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

DOM irradiation, they could not in turn substantially increase BP. The photodegradation of DOM collected in the DCM of GYR inhibited BP by 40% (Fig. 5). This decrease could be related to the photochemical production of biorefractory compounds (Kieber et al., 1997). DOM irradiation in UPW resulted no significant difference in BP for both surface and DCM samples (Fig. 5). This absence of response may be attributed the high amount of carbon initially present in the upwelling system, i.e. heterotrophic bacteria were not limited in carbon before DOM photodegradation. These contrasted bacterial responses to DOM photodegradation reflect differences in the nature and chemical composition of DOM before irradiation (Moran and Covert, 2003).

4 Summary and Conclusion

This study showed a diversity of TDNS amounts in the South East Pacific waters with relatively elevated concentrations in the center of the SPG (GYR) and to a lesser extent in the eastern border of the SPG (EGY). However, TDNS make up only 0.5 to 4% of DOC. In the highly productive area of the Chile upwelling (UPW), we observed a strong coupling between primary and heterotrophic bacterial productions resulting high turnover of labile DOM which may explain the relatively low yields of TDNS. There was no noticeable effect of solar radiation on DOM bacterial assimilation in such environment probably because of the high water mixing and the absence of carbon limitation. We found that in GYR, TDNS were accumulated with respect to the low primary and heterotrophic bacterial productions, and reinforce the idea of the bacterial limitation by nitrogen (Van Wambeke et al., 2007). Such feature is consistent with the DOC accumulation (Raimbault et al., 2007) and likely with the relatively old character of the surface DOM revealed by its elevated C/N ratios (Raimbault et al., 2007). This DOM, accumulated in the surface waters of GYR, does not seem to be photoreactive as indicated by the photo (bio)degradation experiments. Indeed, it has been hypothesized that DOM in this area was continuously photobleached in the surface waters due to the high level of stratification and to high surface UV irradiances (Morel et al., 2007). Other

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

stations, EGY and MAR, showed intermediate characteristics with moderate TDNS concentrations and turnover regarding bacterial and primary productions.

Acknowledgements. We are grateful to the captain and crew of the RV “Atalante” for excellent service during the BIOSOPE cruise. We acknowledge H. Claustre and A. Sciandra as chief scientists of the cruise, as well as C. Bournot, D. Taillez and D. Merien for CTD operations. We also thank P. Raimbault for providing Chl-*a* and DOC data, and T. Moutin and S. Duhamel for providing primary production data. K. Mopper gave a substantial help to our group for improving the quality of the desalination procedure. This research was funded by CNRS-PROOF projects (BIOSOPE and UVECO), and the regional council of Provence Alpes Côte d’Azur.

References

Aluwihare, L. I., Repeta, D. J., and Chen, R. F.: A major biopolymeric component to dissolved organic carbon in seawater, *Nature*, 387, 166–167, 1997.

Amon, R. M. W. and Benner, R.: Combined neutral sugar as indicators of the diagenetic state of dissolved organic matter in the Arctic Ocean, *Deep-Sea Res. I*, 50, 151–169, 2003.

Blain, S., Bonnet, S., and Guieu, C.: Dissolved iron distribution in the tropical and sub tropical South Eastern Pacific, *Biogeoscience Discuss.*, 4, 2845–2875, 2007.

Benner, R. and Kaiser, K.: Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter, *Limnol. Oceanogr.*, 48, 118–128, 2003.

Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I., and Hatcher, P.G.: Bulk chemical characteristics of dissolved organic matter in the ocean, *Science*, 255, 1562–1564, 1992.

Benner, R.: Chemical composition and reactivity, in: *Biogeochemistry of Marine Dissolved Organic Matter*, edited by: Hansell, D. and Carlson, C., Academic Press, 59–90, 2002.

Borch, N. H. and Kirchman, D. L.: Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD, *Mar. Chem.*, 57, 85–95, 1997.

Burney, C. M. and Sieburth, J. M.: Dissolved carbohydrates in seawater. II, A spectrometric procedure for total carbohydrate analysis and polysaccharide estimation, *Mar. Chem.*, 5, 15–28, 1977.

Carr, M. E.: Estimation of potential productivity in Eastern Boundary currents using remote sensing, *Deep-Sea Res. II*, 49, 59–80, 2002.

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

- Claustre, H. and Maritorena, S.: The many shades of ocean blue, *Science*, 302, 1514–1515, 2003.
- Claustre, H., Sciandra, A., and Vaultot, D.: Introduction to the special section: bio-optical and biogeochemical conditions in the South East Pacific in late 2004 – the BIOSOPE program, *Biogeosciences Discuss*, in press, 2008.
- Kieber, R. J., Hydro, L. H., and Seaton P. J.: Photooxydation of triglycerides and fatty acids in seawater: implication toward the formation of marine humic substances, *Limnol. Oceanogr.*, 42, 1454–1462, 1997.
- Kieber, R. J., Zhou, X., and Mopper, K.: Formation of carbonyl compounds from UV-induced photodegradation of humic substances in natural waters: Fate of riverine carbon in the sea, *Limnol. Oceanogr.*, 35, 1503–1515, 1990.
- Kirchman, D. L., Meon, B., Ducklow, H. W., Carlson, C. A., Hansell, D. A., and Steward, G.: Glucose fluxes and concentrations of dissolved combined sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep-Sea Res. II*, 48, 4179–4197, 2001.
- Mopper, K. and Kieber, D. J.: Photochemistry and the cycling of carbon, sulphur, nitrogen and phosphorus, in: *Biogeochemistry of marine dissolved organic matter*, edited by: Hansell, D. A. and Carlson C., A., Academic Press, San Diego, 455–507, 2002.
- Mopper, K., Schultz, C., Chevolut, L., Germain, C., Revuelta, R., and Dawson, R.: Determination of sugars in unconcentrated seawater and other natural waters by liquid chromatography, *Environ. Sci. Technol.*, 26, 133–137, 1992.
- Moran, M. A. and Covert, J. S.: Photochemically mediated linkages between dissolved organic matter and bacterioplankton, in: *Aquatic ecosystems: interactivity of dissolved organic matter*, edited by: Findlay, S. E. G., Sinsabaugh, R. L., Academic Press, San Diego, 243–262, 2003.
- Moran, M. A. and Zepp R. G.: Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter, *Limnol. Oceanogr.*, 42, 1307–1316, 1997.
- Morel, A., Gentili, B., Claustre, H., Babin, M., Bricaud, A., Ras, J., and Tieche F.: Optical properties of the “clearest” natural waters, *Limnol. Oceanogr.*, 52, 217–229, 2007.
- Painter, T. J.: Algal polysaccharides, in: *Polysaccharides*, edited by: Aspinall, G. O., Academic., 195–285, 1983.
- Pakulski, J. and Benner, R.: Abundance and distribution of carbohydrates in the ocean, *Limnol. Oceanogr.*, 39, 930–940, 1994.
- Panagiotopoulos, C. and Sempéré, R.: Molecular distribution of carbohydrates in large marine

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

- particles, *Mar. Chem.*, 95, 31–49, 2005.
- Panagiotopoulos, C., Sempéré, R., Lafont, R., and Kerhervé, P.: Effects of temperature in the determination of monosaccharides by using an anion-exchange column, *J. Chromatogr.*, 920, 13–22, 2001.
- 5 Parsons, T. R., Takahashi, M., and Hargrave, B.: Biological oceanographic processes, Pergamon, 1984.
- Raimbault, P., Garcia, N., and Cerutti, F.: Distribution of inorganic and organic nutrients in the South Pacific Ocean – evidence for long-term accumulation of organic matter in nitrogen-depleted waters. *Biogeosciences Discuss.*, 4, 3041–3087, 2007,
- 10 <http://www.biogeosciences-discuss.net/4/3041/2007/>.
- Rich, J. H., Ducklow, H. W., and Kirchman, D. L.: Concentrations and uptake of neutral monosaccharides along 140° W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux, *Limnol. Oceanogr.*, 41, 595–604, 1996.
- Rich, J. H., Gosselin, M., Sherr, E., Sherr, B., and Kirchman, D. L.: High bacterial production,
- 15 uptake and concentrations of dissolved organic matter in the central Arctic Ocean, *Deep-Sea Res. II*, 44, 1645–1663, 1997.
- Signorini, S. R., McClain, C. R., and Dandonneau, Y.: Mixing and phytoplankton bloom in the wake of the Marquesas Islands, *Geophys. Res. Lett.*, 26, 3121–3124, 1999.
- Skoog, A. and Benner, R.: Aldoses in various size fractions of marine organic matter: Implications for carbon cycling, *Limnol. Oceanogr.*, 42, 1803–1813, 1997.
- 20 Skoog, A., Biddanda, B., and Benner, R.: Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico, *Limnol. Oceanogr.*, 44, 1625–1633, 1999.
- Smith, D. C. and Azam, F.: A simple, economical method for measuring bacterial protein synthesis rates in sea water using 3H-Leucine, *Mar. Microb., Food Webs*, 6, 107–114, 1992.
- 25 Stouthamer, A. H.: Energetic aspects of the growth of micro-organisms, *Symp. Soc. Gen. Microbiol.*, 27, 285–315, 1977.
- Tedetti, M., Sempéré, R., Vasilkov, A., Charrière, B., Nérini, D., Miller, W. L., Kawamura, K., and Raimbault, P.: High penetration of ultraviolet radiation in the south east Pacific waters, *Geophys. Res. Lett.*, 34, L12610, doi:10.1029/2007GL029823, 2007.
- 30 Thingstad, T. F., Hagstrom, A., and Rassoulzadegan, F.: Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop?, *Limnol. Oceanogr.*, 42, 398–404, 1997.
- Van Wambeke, F., Bonnet, S., Moutin, T., Raimbault, P., Alarçon G., and Guieu, C.: Factors

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

limiting heterotrophic bacterial production in the southern Pacific Ocean, Biogeosciences Discuss., 4, 3799–3828, 2007,

<http://www.biogeosciences-discuss.net/4/3799/2007/>.

- 5 Van Wambeke, F., Obernosterer, I., Moutin, T., Duhamel, S., Ulloa, O., and Claustre, H.: Heterotrophic bacterial production in the South East Pacific: Longitudinal trends and coupling with primary production, Biogeosciences, 5, 1–13, 2008a.
- Van Wambeke, F., Tedetti, M., Duhamel, S., and Sempéré, R.: Diel variability of heterotrophic bacterial production and UV doses in the South East Pacific, Biogeosciences Discuss., 5, 435–462, 2008b.

10

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

Sugars in the South Pacific

R. Sempéré et al.

Table 1. Site location, date of sampling, deep chlorophyll maximum (DCM), depth of the euphotic zone (Z_{eu} , 1% surface PAR), temperature (T), salinity, chlorophyll-*a* (Chl-*a*) concentration, and bacterial and primary productions integrated down to DCM (I-BP and I-PP) at the four stations in the South East Pacific.

Site	Latitude	Longitude	Date	DCM (m)	Z_{eu} (m)	T (°C)		Salinity		Chl- <i>a</i> ($\mu\text{g l}^{-1}$)		I-BP ($\text{mg C m}^{-2} \text{ d}^{-1}$)	I-PP ($\text{mg C m}^{-2} \text{ d}^{-1}$)
						5 m	DCM	5 m	DCM	5 m	DCM		
MAR*	08°21'S	141°16'W	27 ^a , 28 ^b , 29 ^c Oct. 04	60	70	27.5	27.5	35.6	35.6	0.32	0.43	143	683
GYR	26°03'S	114°00'W	12 ^b , 14 ^a , 15 ^c Nov. 04	175	160	23	15	36	35.6	0.03	0.19	50	159
EGY	31°50'S	091°27'W	26 ^b , 28 ^{a,c} Nov. 04	80	88	18	16	34.7	34.6	0.07	0.21	54	196
UPW	34°01'S	073°21'W	06 ^{a,c} , 07 ^b Dec. 04	40	34	15.5	14.5	34.2	34.2	0.21	0.72	226	4362

* At 160 m, which is the depth of the second DOM-photodegradation and -biodegradation experiment, $T=25^{\circ}\text{C}$, salinity=36.0 and $\text{Chl-}a=0.33 \mu\text{g l}^{-1}$.

^a Sampling for dissolved neutral sugar and BP profiles (bio-optical CTD cast, close to solar noon).

^b Sampling for DOM-photodegradation and -biodegradation experiments (00:00 CTD cast). Surface solar irradiance was measured continuously during the exposure period (~8 h around solar noon).

^c Underwater solar irradiance measurements (close to solar noon).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

I◀

▶I

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Sugars in the South Pacific

R. Sempéré et al.

Table 2. 10% irradiance depths ($Z_{10\%}$) in the UVB (305 nm), UVA (325, 340, 380 nm) and visible (412, 443, 490, 565 nm) spectral domains at the four stations in the South East Pacific.

	$Z_{10\%}$ (m)							
Site	305 nm	325 nm	340 nm	380 nm	412 nm	443 nm	490 nm	565 nm
MAR	9.6	17.1	23.3	37.0	39.2	41.9	49.2	26.8
GYR	21.4	40.4	57.4	108.9	107.0	113.1	97.4	36.5
EGY	11.8	22.7	28.3	53.4	47.1	49.0	54.4	27.4
UPW	3.0	4.9	5.8	8.8	9.9	10.6	14.5	17.8

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[I◀](#)
[▶I](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

Sugars in the South Pacific

R. Sempéré et al.

Table 3. Doses (surface irradiances integrated over time) received by the DOM-solutions during the photodegradation experiments in the UVB (305 nm), UVA (325, 340, 380 nm) and visible (412, 443, 490, 565 nm) spectral domains at the four stations in the South East Pacific.

		Dose (kJ m ⁻²)							
Site	Sky	305 nm	325 nm	340 nm	380 nm	412 nm	443 nm	490 nm	565 nm
MAR	sunny	2.2	11.9	16.2	22.9	36.8	41.6	47.0	43.9
GYR	cloudy	1.4	7.7	10.5	14.5	22.9	25.1	27.5	25.2
EGY	cloudy	1.3	7.4	10.1	13.8	21.8	23.9	26.2	23.8
UPW	sunny	2.5	13.5	18.4	25.9	41.3	46.6	52.7	49.0

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Sugars in the South Pacific

R. Sempéré et al.

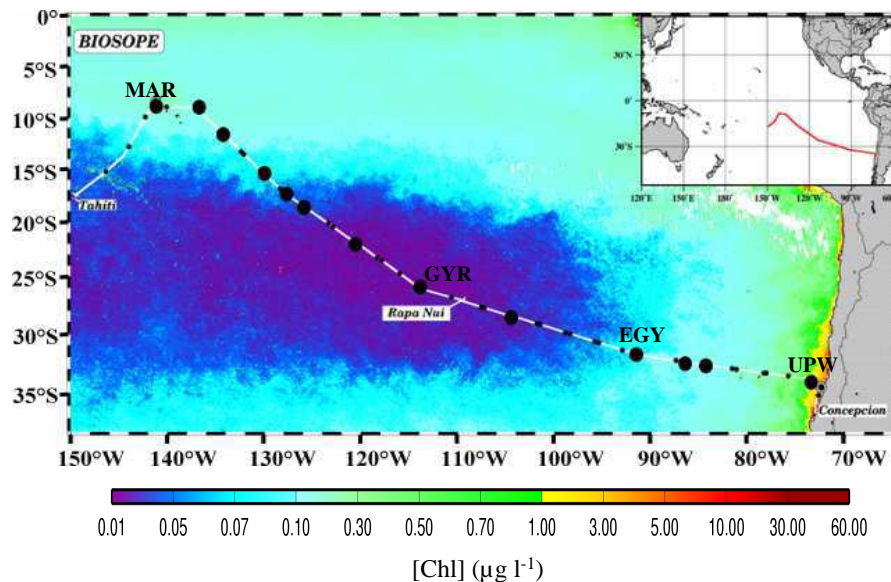


Fig. 1. Map of the BIOSOPE cruise track superimposed on a Sea-viewing Wide Field-of-view Sensor (SeaWiFS) composite for November and December showing the chlorophyll concentration in the upper layer. The long (3–6 days) stations studied for dissolved neutral sugar content, bacterial production, DOM-photodegradation and -biodegradation experiments are Marquesas Islands (MAR), center of the South Pacific Gyre (GYR), East of the South Pacific Gyre (EGY) and upwelling off Chile (UPW) (indicated with a star). Rapa Nui is Easter Island.
<http://www.obs-vlfr.fr/proof/vt/op/ec/biosope/bio.htm>.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

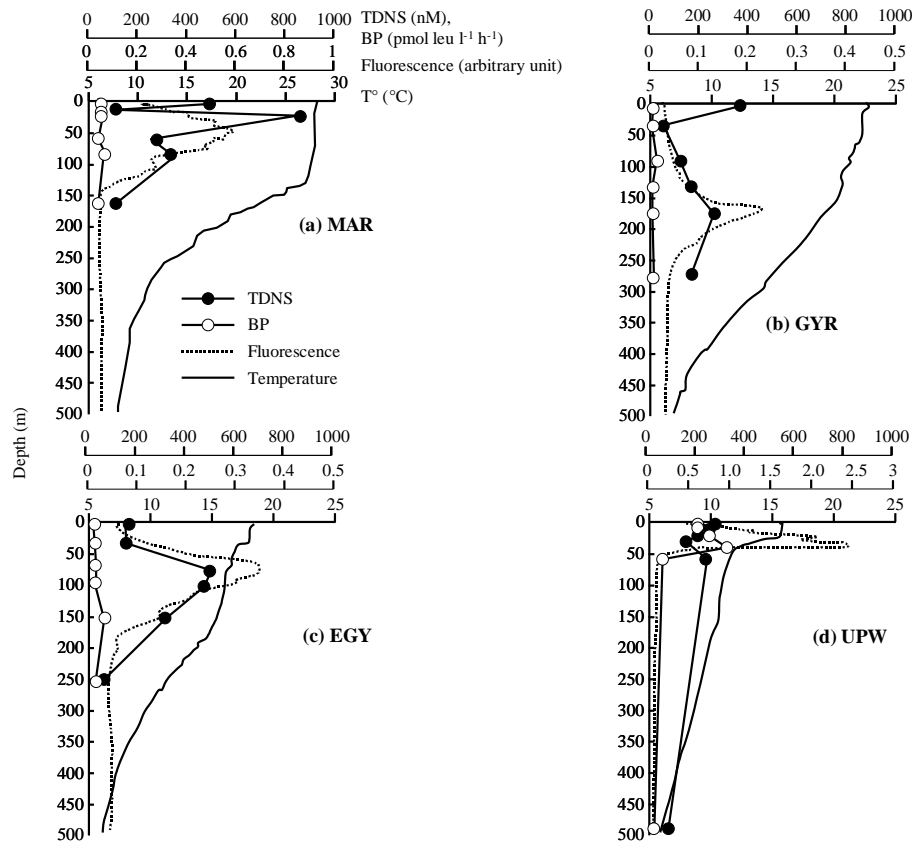


Fig. 2. Depth profiles of total dissolved neutral sugars (TDNS in nM), bacterial production (BP in pmol leu l⁻¹ h⁻¹), chlorophyll fluorescence (arbitrary unit), and temperature (°C) at the four stations in the South East Pacific.

BGD

5, 725–750, 2008

Sugars in the South Pacific

R. Sempéré et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

EGU

Sugars in the South Pacific

R. Sempéré et al.

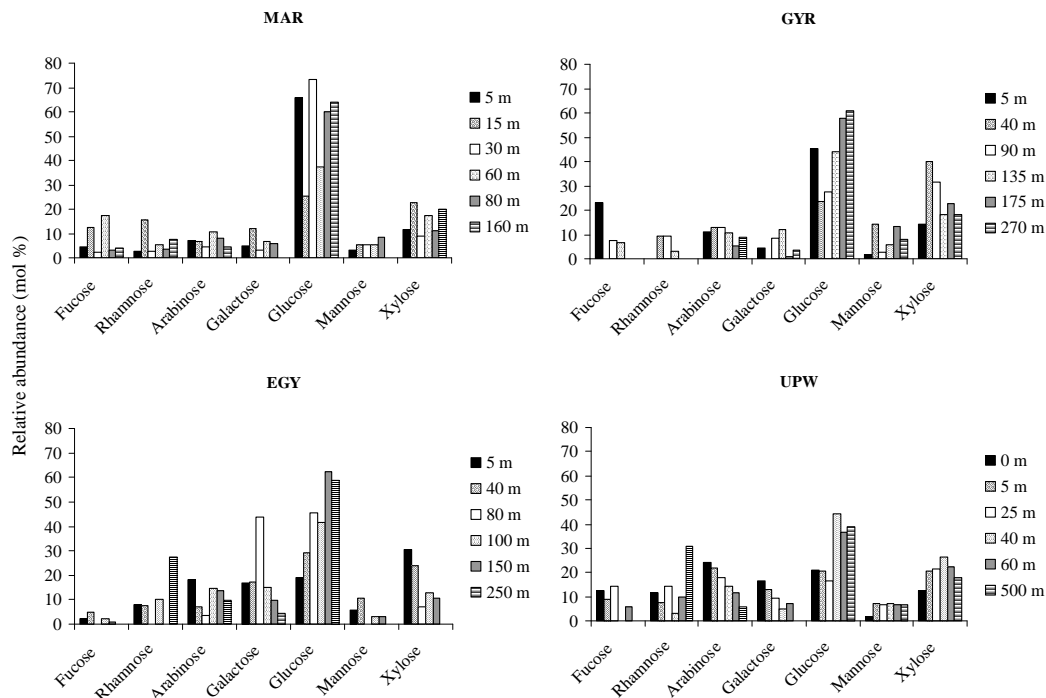


Fig. 3. Relative abundance of total dissolved neutral sugars (TDNS in mol %) at the four stations in the South East Pacific.

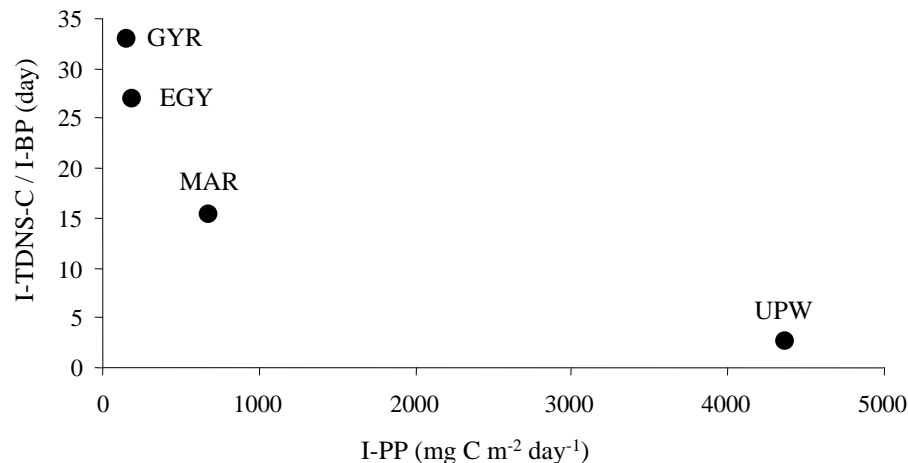


Fig. 4. Relationship between I-TDNS-C/I-BP ratios [total dissolved neutral sugars in carbon integrated down to DCM (I-TDNS-C in mg C m⁻²) and bacterial production integrated down to DCM (I-BP in mg C m⁻² day⁻¹)] and primary production integrated down to DCM (I-PP in mg C m⁻² day⁻¹) at the four stations in the South East Pacific.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Sugars in the South Pacific

R. Sempéré et al.

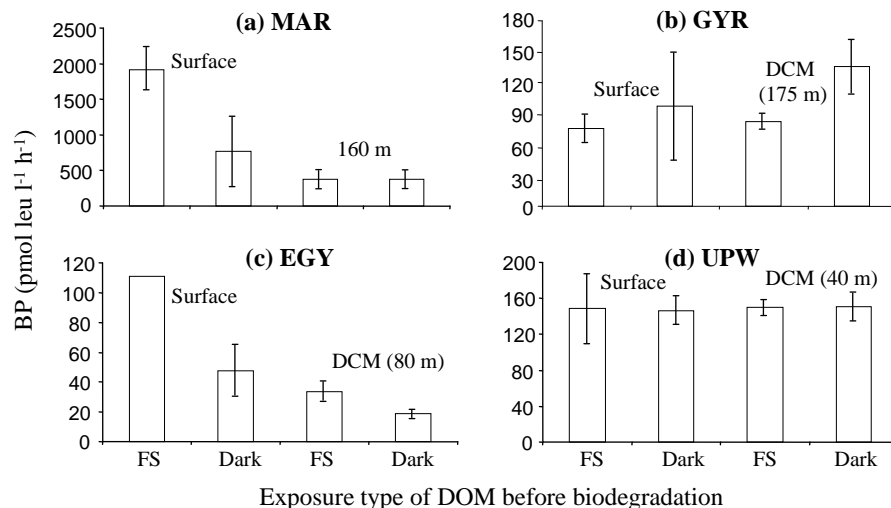


Fig. 5. Bacterial production (BP in $\text{pmol leu l}^{-1} \text{h}^{-1}$) after 48 h (MAR) or 72 h (GYR, EGY, UPW) incubation of irradiated DOM-solution ($0.2\text{-}\mu\text{m}$ filtered seawater) with a bacterial inoculum ($0.8\text{-}\mu\text{m}$ filtered seawater, 1/6, inoculum/DOM-solution final ratio) at the four stations studied in the South East Pacific. Samples were collected at two depths, i.e. 5 m and DCM, except in MAR (5 and 160 m). DOM-solutions were irradiated under solar radiation (~ 8 h around solar noon) in full sun (FS) or kept in the dark (Dark). Standard deviations are based on duplicate samples.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

I◀

▶I

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion